



## Short communication

## Embryo production and recovery in goats by non-surgical transcervical technique

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## ABSTRACT

The objective of this study was to evaluate the efficiency of non-surgical embryo collection in goats. Five lactating Saanen goats were tested alternately in a cross-over design for two treatments (T1 and T2). In both treatments, intravaginal progesterone devices were inserted (day 0/6 p.m.) and removed six days later (day 6/18:00 p.m.) and 22.5 µg cloprostenol were administered by intravulvosubmucosal via (day 0). From day 4 (18:00 p.m.), goats received six decreasing doses of FSH (250–400 IU) at 12 h interval. Three doses of 50 mg flunixin meglumine were administered on days 9, 10, and 11. In T1 and T2, animals received two doses of saline or 250 mg of rbST on day 0 and on first detection of estrus, respectively. Goats were mated until the end of estrus and uteri were flushed by cervical route seven days after first mating. The embryo collection interval was 60 days. Percentage of flushing medium recovered was close to 100% in both treatments. Total number of ova ( $18.4 \pm 9.4$  and  $15.7 \pm 11.0$ ) and viable embryos ( $13.4 \pm 4.1$  and  $8.2 \pm 3.0$ ) were similar ( $P > 0.05$ ) for T1 and T2, respectively. Short-term progesterone protocol was efficient for superovulation in lactating Saanen goats but association with rbST did not enhance embryo recovery or

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## 1. Introduction

The first successful embryo transfer was reported in rabbits by Heape in 1890. Since then, embryo transfer (ET) procedures were performed in many domestic animal species. Warwick et al. (1934) reported the first goat kid generated by ET techniques. In Brazil, successful ET in goats was first reported by Jaume and Bruschi (1985). Nevertheless, the commercial use of these techniques was only reported in the 70s, in North America (Jainudeen et al., 2004). All ET reports in goats until 1990 were based on surgical procedures. Since then, most ET reports in goats

were based on surgical procedures, and the unavailability of non-surgical techniques limited the use of ET in some countries (Anon, 1994).

The low economic value of most sheep and goats and the invasive procedures required make ET less useful in small ruminants than in cattle. Post-surgical sequels, such as adhesions, and consequent concerns about animal welfare also limited the widespread use of ET in small ruminants (Mobine et al., 2002). Therefore, it was expected that non-surgical techniques would replace the current surgical procedures for in vivo embryo production in these species, just like occurred in cattle. However, the relatively small size of the reproductive traits of sheep and goats and consequent difficulty in manipulation, and the lack of appropriated instruments, delayed the development of the non-surgical approach. Pereira et al. (1998) reported the

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possibility of recovering goat embryos by cervical route. Nowadays, most of embryo recovery in Brazilian goat herd is made by non-surgical cervical flushing, and there are great efforts to adapt it also to sheep (Gusmão et al., 2007, 2009).

Another important problem to in vivo embryo production in small ruminants is the great variability of the superovulation process. It was previously demonstrated that superovulatory responses can be improved if FSH treatment is begun close to follicular wave emergence, aiming to avoid the deleterious effect of follicle dominance (characterized by the presence of follicles larger than 5–6 mm diameter) (Fonseca et al., 2010). The association of bovine somatotropin (bST) with FSH in the superovulatory protocols improved embryo yield cattle (Moreira et al., 2002) and sheep (Montero-Pardo et al., 2011), but was not evaluated in goats yet.

The objective of this study was to describe the non-surgical embryo collection technique and evaluate its efficiency in goats undergoing different superovulation protocols.

## 2. Materials and methods

### 2.1. Location

This study was carried out during the local non breeding to transition season (November–March) in Coronel Pacheco, Minas Gerais State, South-east region of Brazil. The research unit is located at 435 m altitude, 21° 35' S and 43° 15' W latitude and longitude, respectively. The average annual precipitation was 1581 mm<sup>3</sup>. Average annual temperature experienced at site is 21 °C.

### 2.2. Animals and management

This study was approved by Ethics and Animal Care Committee (CEUA) from Fluminense Federal University on August 25th, 2011. Five lactating Saanen goats were used in this study. The mean body weight and condition score (1–5 variations) were 76.8 ± 4.7 kg and 3.8 ± 0.2, respectively. Animals were confined and received corn silage and a protein concentrate according to their maintenance demand. Mineral salt and water were offered ad libitum.

### 2.3. Experimental design and treatments

A cross-over design was used, with animals being tested alternately in two treatments (T1 and T2). In all animals, intravaginal progesterone devices (CIDR®; Pfizer Animal Health, São Paulo, Brazil) were inserted (day 0, 06:00 p.m.) and removed six days later (day 6), and 30 µg d-cloprostenol (Prolise®, ARSA S.R.L., Buenos Aires, Argentina) was injected intravulvo-submucosal (day 0, 18:00 p.m.). Beginning on day 4 (06:00 p.m.), goats received six decreasing doses (25–25–15–15–10–10%) of FSH (Pluset®, Hertape Calier, São Paulo, Brazil) in a proportion of 4 IU/kg body weight (min 250 and max 400 IU) at 12 h interval (06:00 a.m. and 06:00 p.m.). Three doses of 50 mg flunixin meglumine (Banamine®, Shering Plough, São Paulo, Brazil) were injected (06:00 a.m.) on days 9, 10, and 11. Animals received two doses of saline (T1) or 250 mg of rbST (T2; Lactotropin®, Elanco, São Paulo, Brazil) on day 0 and on first estrous detection, respectively. Estrous detection and mating was done twice daily, starting 12 h after device removal until the end of estrus. The goats were considered to be in estrus when they stood to be mounted by two 4-yr-old bucks. The embryo collection interval was 60 days. One of the goats showed estrus one day after flushing. It was mated and submitted to embryo collection again seven days after estrus.

### 2.4. Donor preparation

All donor goats underwent non-surgical uterine flushing by cervical route. Embryo recovery was done six to seven days after first breeding. Goats received 30 µg d-cloprostenol (Prolise®, ARSA S.R.L., Buenos

Aires, Argentina) 12 h before flushing. The hair from the tail insertion was shaved. The perineal region was washed with clean water and detergent. Special attention was given to total removal of residual fecal material on anus and vulva. No alcohols based solutions were used on these superficialities.

### 2.5. Sedation and anesthesia

Ten minutes before onset of flushing, goats receive 1% acepromazine (Acepran 1%®, Vetinil/Univet, São Paulo Brazil) solution in a proportion of 1 mL/100 kg BW i.m. Immediately before vaginal speculum introduction, 1–2 mL of lidocaine hydrochloride 2% without vasoconstrictor were administered per animal by epidural route in the sacrococcygeal region.

### 2.6. Cervical immobilization and traction

A 2–3 Collin speculum was inserted to explore vagina and cervix of the goats. Eventually the speculum size was changed according to the facility to view and expose the cervix.

Gel was used to lubricate the frontal edge of the speculum, which was introduced into vulva/vestibule and vagina slowly. A light source aided to maintain speculum in a central position, when it was opened with attention to cervical exposition. At this time, the female usually contracted the abdomen. As a consequence, cervix projected into speculum center. Two 26 cm Pozzi forceps tweezers were clipped 0.5–1 cm laterally to cervical opening. After cervix immobilization, with the aid of Allis forceps (26 cm), sterile gauze soaked with 5 mL lidocaine 2% without vasoconstrictor was gently introduced ventrally to cervical opening. The gauze was kept in this place during the procedure and removed at the end.

### 2.7. Cervical transposition

Hegar uterine dilators # 3 to 4 were used to transpose cervical rings. At this stage, the first rings could be transposed, and further movement across the cranial cervical rings was aided by rectal manipulation using one finger. After insertion of Hegar dilator into cervical opening, a gentle rotation and forward movement was needed to move it slowly through the cervix, traversing cervical rings. After total transposition, Hegar dilator was kept in place for 30 s. Then, a caprine/ovine specially developed sterile catheter number 10 (Sonda Embrapa® for goat/sheep embryo recovery; Embrapa, Brasília, Brazil) equipped with a metal mandrel was used to bypass the cervical rings again. Resistance was lost after total transposition of rings. After that, cervix was slightly tract back and the catheter was guided to the desired uterine horn by rectal finger manipulation.

### 2.8. Embryo recovery

A specially developed circuit was connected to catheter (Circuito Embrapa® for goat/sheep embryo recovery; Embrapa, Brasília, Brazil). The circuit is a fully closed system consisting of transparent, non-toxic, sterile and flexible vinyl tubes assembled to allow two ways of communication with the catheter, both connected in a Y shape. One of the circuit lines communicates with the embryo collection filter and the other was connected to the hose linked to the washing medium (similar to the one used in cattle). The circuit also has a three way device near to extremity connected to the washing medium. A 60 mL syringe was inserted in this device to control the volume of liquid instilled in each uterine horn per time, usually fractions of 15 mL. During each washing procedure, the route attached to the filter was temporarily closed by a flow blocker and the route linked to the catheter was opened, so that, the washing medium was instilled in each uterine horn. In the sequence, the route attached to the filter was opened allowing the flushing medium to return from the uterus. When liquid was instilled toward the uterine horn, both communications to washing medium and filter were closed. A total of 180 mL of medium was used to flush each uterine horn. In some cases and animals, mainly in pluriparous, the amount of liquid per time was increased to 20 mL.

The last procedures included careful removal of Pozzi forceps and gauge. All the processes were carried out with animal in standing position and contained in an appropriate box that avoids lateral and dorsoventral movements.

**Table 1**

Effects of short-term superovulation with or without association of bovine somatotropin (bST) in Saanen goats submitted to non surgical embryo collection by transcervical route (m ± SE).

Variable	Control goats	bST goats
Number of goats	5	5
Estrous response (%)	100	100
Interval to estrus (h)	31.2 ± 6.1	24.4 ± 4.5
Number of mating	3.0 ± 0.3	3.8 ± 0.6
Collection duration (min)	35.0 ± 3.9	34.0 ± 2.7
Flushing recovered (%)	97.3 ± 1.0	96.4 ± 1.3
Viable embryos	13.4 ± 4.1	8.2 ± 3.0
Degenerated embryos	0.4 ± 0.2	2.8 ± 1.7
Unfertilized oocytes	6.0 ± 4.2	4.2 ± 3.7
Total structures	18.4 ± 9.4	15.7 ± 11.0

### 2.9. Variables and statistical analysis

The following parameters were calculated:

Percentage of animals in estrus: [number of females in estrus/number of females treated] × 100;  
Interval to estrus: interval (h) from sponge removal to the time of first estrous identification (onset of estrus);  
Duration of estrus: interval (h) from time of the first to the last estrous identification.  
Fluid flushing efficiency: percentage of fluid recovered per fluid infused;  
Embryo flushing efficiency: percentage of structures recovered per number of corpora lutea detected;  
Embryo morphology and quality;  
Time elapsed from epidural anesthesia from Pozzi forceps removal;  
Parametric variables expressed as mean ± SE were submitted to one-way analysis of variance and compared by Tukey test using a SAEG program (Ribeiro Júnior, 2001).

## 3. Results

The duration of the whole procedure, from speculum insertion into the vagina to the end of flushing, was approximately 30–40 min and the efficiency of flushing medium recovery was superior to 95%, as shown in Table 1. All parameters studied were similar ( $P > 0.05$ ) between treatments.

One goat presented 27 (8 embryos and 19 unfertilized) and 26 (4 embryos and 22 unfertilized) ova for T1 and T2, respectively. The goat that showed estrous behavior and was mated one day after embryo collection underwent embryo collection seven days later resulting in three excellent blastocysts. No apparent lesions or sequels were noted in the reproductive tract.

## 4. Discussion

Both superovulation protocols were efficient to stimulate ovarian response, resulting in good embryo yield. The total estrous response (100%), interval to estrus, and number of mating were superior or similar to previous study reported in goats (Greyling et al., 2002). These results leaved to a good expectative for embryo collection.

The non surgical technique used in present study was described in detail as never reported before. Although data presented in this study was supported by limited observations ( $n = 11$ ), the non-surgical technique has been used in hundreds of goats in our routine practice (Fonseca, personal data). It therefore warrants that the technique

can be replicated in goats worldwide. The duration of the procedure (20–40 min) showed to be equivalent to those reported (less than 45 min) previously (Suyadi et al., 2000). The standing position and the relative easy sedation and anesthesia are great differentials from the surgical approach. The possibility of recovering embryos from a goat eight days after a previous flushing after a superovulation protocol suggests that the procedure used in the present study caused no significant lesions or sequels in the reproductive tract and also opens the possibility of recovering embryos from animals not previously stimulated by FSH. Thus, animals showing no response to exogenous FSH, older or special females also underwent a non-surgical embryo collection. In standard conditions, these kind of animals would not be used as embryo donors.

Despite being one of the fastest growing sectors in the livestock market in Brazil, the goats and sheep breeding still lack advanced reproductive technologies developed for these species. Currently, embryo recovery from small ruminants uses mostly adapted materials from other species. In this scenario, the development of specific equipment for their anatomical features can consolidate the transcervical procedure as the technique of choice for embryo recovery in these animals, opening a promising market to be exploited. Success regarding to flushing medium recovered was reported in sheep (95%; Gusmão et al., 2007) and in lower scale (80%) in goat (Suyadi et al., 2000). In the present study, both sonda and circuit showed great efficiency, as demonstrated for the first time by the recovery of more than 95% of flushing medium in a true completely closed circuit. This fact supports the expectation of improved efficient in embryo recovery, reduction in contamination of both flushing medium and donor, and finally encourages the expansion in the use of non surgical embryo recovery techniques.

Despite not being measured in the present study, considering the total number of structures recovered, we can suppose that embryo recovery rate was as efficient as in surgery procedures (Greyling et al., 2002). In the present study, three embryos were collected from a donor without FSH regime, suggesting the efficiency of the transcervical technique. Furthermore, in a transgenic goat, using laparoscopic evaluation, we observed that recovery rate was 69.2% (9/13) and 72.7% (8/11), using surgical and transcervical method, respectively (Moura et al., 2011).

The short-term protocol used in this study was based in a previous work in goats with induced estrous (Maffili, 2004). The results of that study showed that when treated with short-term synchronization using CIDR for five days plus prostaglandin administration on CIDR insertion, goats experienced follicular regression and a new follicular emergence 3.5 days after CIDR insertion. Thus, initializing FSH administration 4.5 days after device insertion we expected to avoid dominant follicles and their deleterious effect on superovulatory response. As shown in Table 1, our hypothesis was confirmed considering the excellent total response for both treatments and excellent and good response in viable embryos for control and bST treatments, respectively.

Finally, the priming (Joyce et al., 1998) and concomitant (Montero-Pardo et al., 2011) use of bST in addition

to superovulatory protocol in sheep have reported no additional increase in ovulation rate. In function of limited observations in the present study, the increase of number of donors stimulated by FSH/bST association should be investigated in goats.

## 5. Conclusions and perspectives

The short-term superovulation protocol used in the present study was adequate for Saanen donor goats. In addition, the embryo recovery technique described is simple, less time consuming, provide the possibility for successive embryo recovery in goat with relatively few health risks and sequels. Using non-surgical technique, females with expected embryo yield close to the natural ovulation rate (less than five ova) can be used in embryo transfer programs. The actual world scenario of assisted reproductive technologies and animal well-being will probably imply in reduction or perhaps in the prohibition of successive surgery procedures in the same animal. Like in cattle, it is expected that non surgical recovery of *in vivo* produced embryos in goats, and in some extension in sheep, becomes the technique of choice in commercial activity around the world, as has become in Brazil.

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